TaqMan multiplex PCR of a seven-gene host biomarker to discriminate bacterial from viral infections

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Wensheng Nie[1], David Rawling[1], Nhung Huynh[1], Melissa Remmel[1], Mark Eshoo[1], Purvesh Khatri[2], Jonathan Romanowsky[1], Oliver Liesenfeld[1,*] and Timothy Sweeney[1]
1. Inflammatix Inc, Burlingame, CA, 2. Institute for Immunity, Transplantation and Infections, and Biomedical Informatics Research, Stanford University School of Medicine, Palo Alto, CA, * Corresponding Author

Background

Acute infections are among the most frequent diagnosis in outpatient care settings, but physicians give antibiotics unnecessarily 30% of the time.[1] Early, accurate and rapid differentiation between viral and bacterial infections is critical to guide the choice of antimicrobial treatment, improve patient outcome, and ensure antimicrobial stewardship. Current microbiological offerings rely on direct pathogen detection, which is limited by insufficient accuracy.[2]

Recently, host response-based molecular diagnostics have been considered as a novel alternative or complimentary approach. We have previously developed and validated a 7-gene signature set (higher in viral infections (*IFI27, JUP, and LAX1*) and higher in bacterial infection (*HK3, TNIP1, GPAA1, and CTSB*) that accurately discriminated between viral and bacterial infections (in 6 validation cohorts, summary ROC AUC of 0.91 (95% CI, 0.82 to 0.96).[3,4] To translate the gene set derived from public and private microarray and next generation sequencing (NGS) data using bioinformatics tools into a rapid and easy to use assay for clinical application, we here describe the development of a rapid multiplex test (HostDx™ Fever), a 7-gene host response PCR assay that discriminates bacterial from viral infections. The HostDx Fever test is being developed as a cartridge-based, sample-to-answer, quantitative assay with a turnaround time of less than 30 minutes (**Fig. 1**).

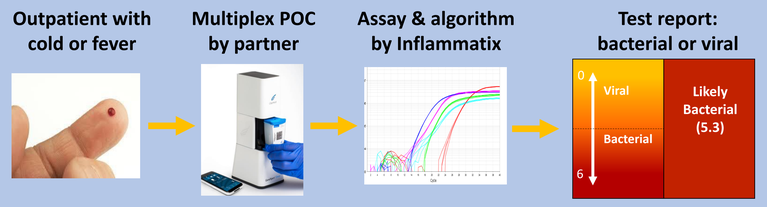

Figure 1. Workflow of HostDx Fever test, sample processing using rapid POC platform, and potential test report.

Methods

To convert the 7-gene set generated using bioinformatics tools into a rapid and easy to use assay for clinical application, we designed multiplex TaqMan® assays to be run on a regular qPCR instrument, QuantStudio™ 6 Flex Real-Time PCR System (QS6™). Assays were optimized for each of the seven targets and validated using TaqPath™ 1-Step Multiplex Master Mix. Templates for qPCR reactions were either RNAs extracted from clinical blood samples collected in PAXgene® blood RNA tubes or synthetic DNAs that mimic the gene expression profile of blood samples. Data was then compared with our internal standard, NanoString nCounter® (amplification-independent gene quantitation platform) and an ultrafast qPCR platform, Puck*dx*™ from The Technology Partnership (TTP), respectively.

Results

7 TaqMan assays were divided into two multiplex reactions, one 5-plex and one 4-plex. KPNA6 was included as housekeeping control in each of the two multiplexes. 9 clinical samples from healthy subjects (3) or patients with confirmed viral (3) or bacterial (3) infections were tested in parallel on QS6 and nCounter platforms. We found a high degree of concordance with R values of >0.95 between QS6 and nCounter platform. More importantly, RNA samples from patients with viral and bacterial infections were well differentiated based on bacterial/viral metascores (difference of geometric means between positive and negative genes[2]) calculated with either Ct values from QS6 or count numbers from nCounter (**Fig. 2**).

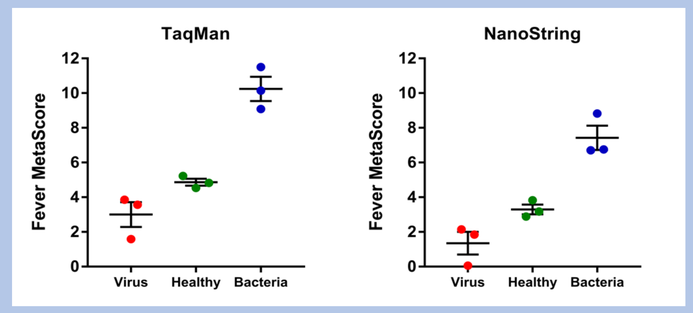

Figure 2. Correlation of results between QS6 and nCounter in 9 RNA samples of patients with confirmed viral or bacterial infections and controls (metascore correlation for 6 gene comparison: R=0.95)

Separately, in order to achieve a time to result that would be more relevant for point of care diagnostics, we evaluated the ultrafast Puck*dx* platform. 8 DNA samples were tested with one 4-plex (KPNA6 included as control) on QS6 and Puck*dx*. ΔCts were highly concordant with an overall R value of >0.94 (**Fig. 3**). Results from Puck*dx* were obtained in less than 15 minutes (compared to ~50 minutes on QS6 and >18 hours on nCounter).

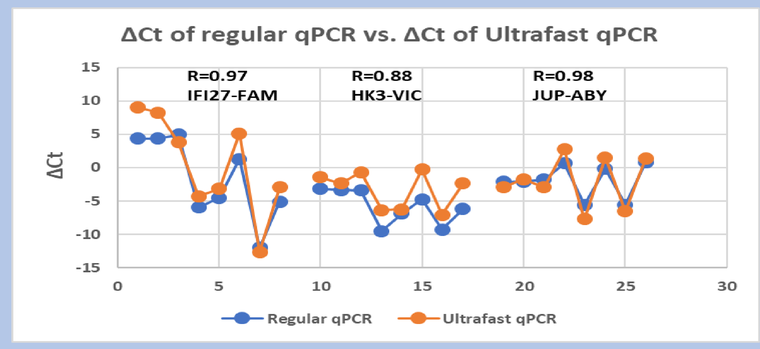

Figure 3. Correlation of results between regular qPCR (QS6) and an ultrafast qPCR platform (Puck*dx*) in 8 DNA samples (ΔCt correlation for 3 gene comparison: R=0.94)

Conclusion

The 7-gene set derived from public and private microarray and NGS data was successfully converted to a Taqman qPCR assay format. An ultrafast qPCR platform generated results in less than 15 minutes. Multiplexing and the ultrafast platform enable HostDx Fever test to be used in clinical practice. As a rapid and highly accurate test, HostDx Fever promises to provide actionable results for improved decision making (antibiotics or not) for patients with acute infections. HostDx Fever results combined with the rapid turnaround time are of great value to assist in the management of patients in outpatient and urgent care settings.

References

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